A Sysmex Group Company

Interpret

NGS Analysis Software

CytoSure™

SureSeq™

Interpret Quick-Start Guide

Oxford Gene Technology

Founded by Professor Sir Ed Southern, Oxford Gene Technology (OGT) supplies world-class genetics research solutions to leading clinical and academic research institutions.

- **CytoSure™** — Class-leading products offering the complete array and NGS solution for clinical genetics research.

- **Cytocell®** — High-quality fluorescence *in situ* hybridisation (FISH) probes for the detection of gene rearrangements related to inherited genetic disease and cancer.

- **SureSeq™** — Hybridisation-based NGS panels and library preparation reagents delivering flexibility and unparalleled coverage uniformity for clinical cancer research applications.

For more information, visit www.ogt.com.

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Upload the FASTQ Files

Note: For each sample to be processed by Interpret, the input is a pair of FASTQ files (one for read 1; one for read 2). However, the sequencer may generate multiple FASTQ files for each sample for each lane on which the sample was sequenced. If this is the case, and you do not know how to combine the FASTQs, please contact OGT for support (support@ogt.com).

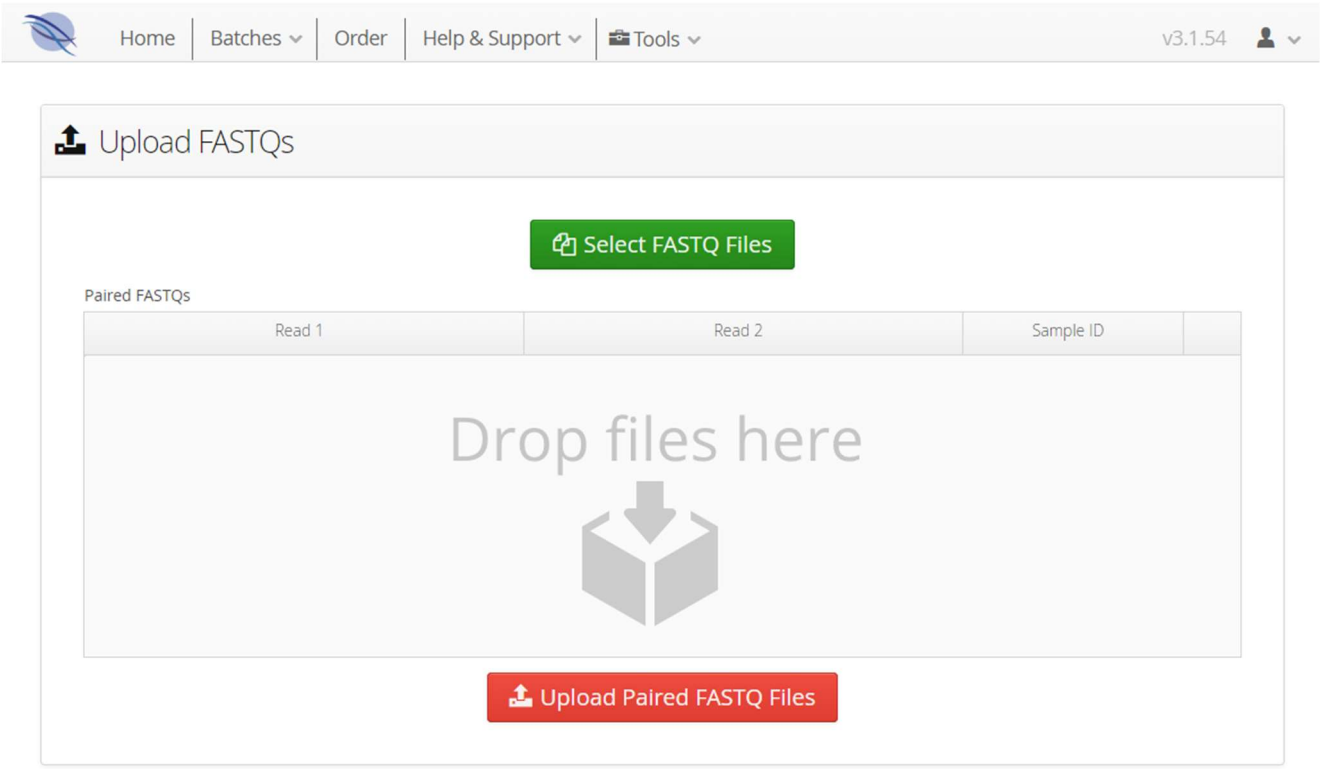
1. Open Interpret using the **Interpret v3** desktop shortcut. If no such link is provided, open a web browser and navigate to the URL of Interpret, which will take the format:

http://COMPUTERNAME:PORT/sureseq

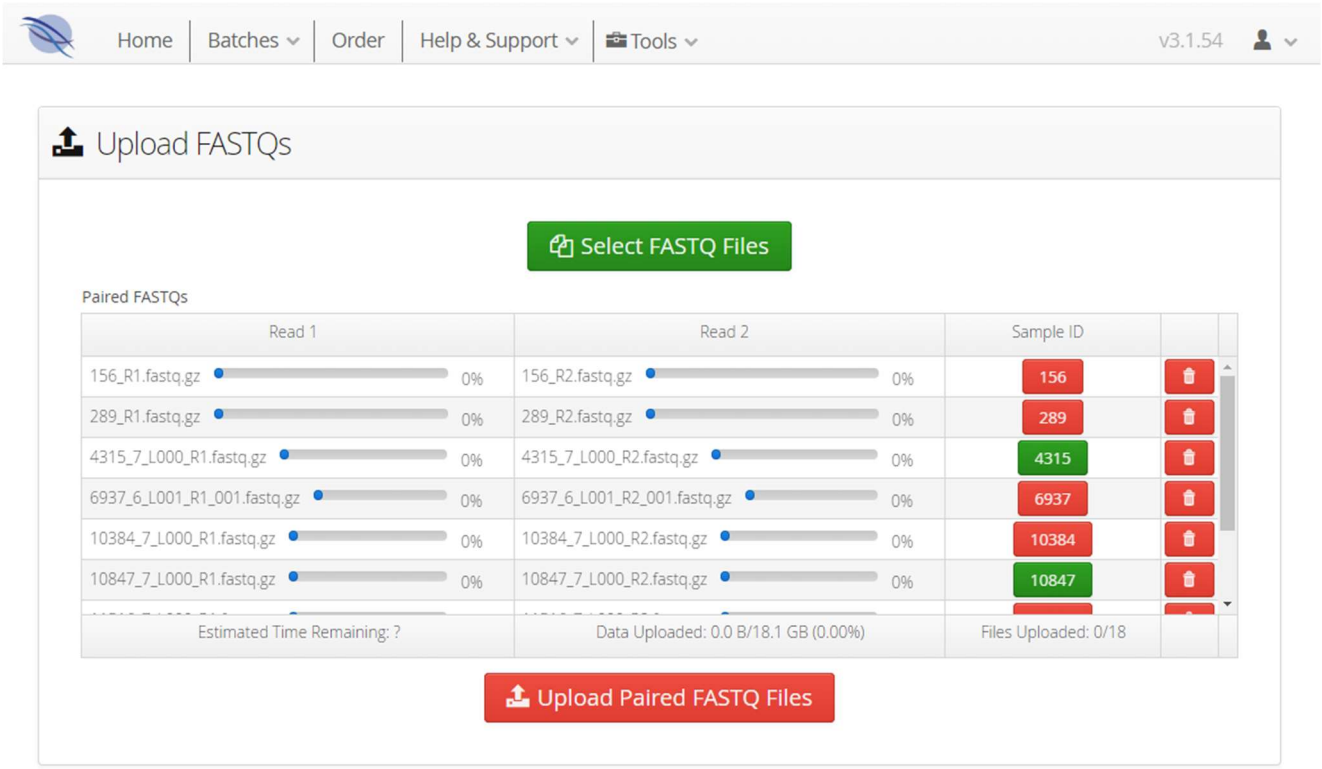
If the software has been installed on the computer in use, but by a different user, the COMPUTERNAME will be "localhost". If it has been installed on another computer on the network, COMPUTERNAME will be the name or IP address of the computer on the network. The default PORT is 80, but may have been changed to something else by the user during installation. A web page similar to the following should be displayed:

2. Login with the user name "admin" and the password provided during installation.

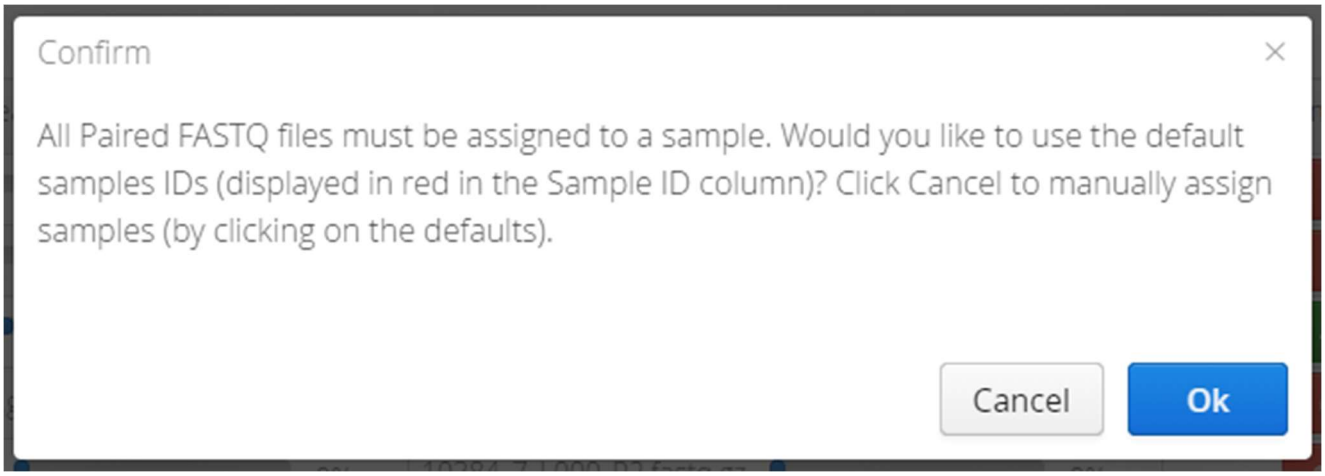
3. Click the **Upload FASTQs** button at the bottom of the screen.



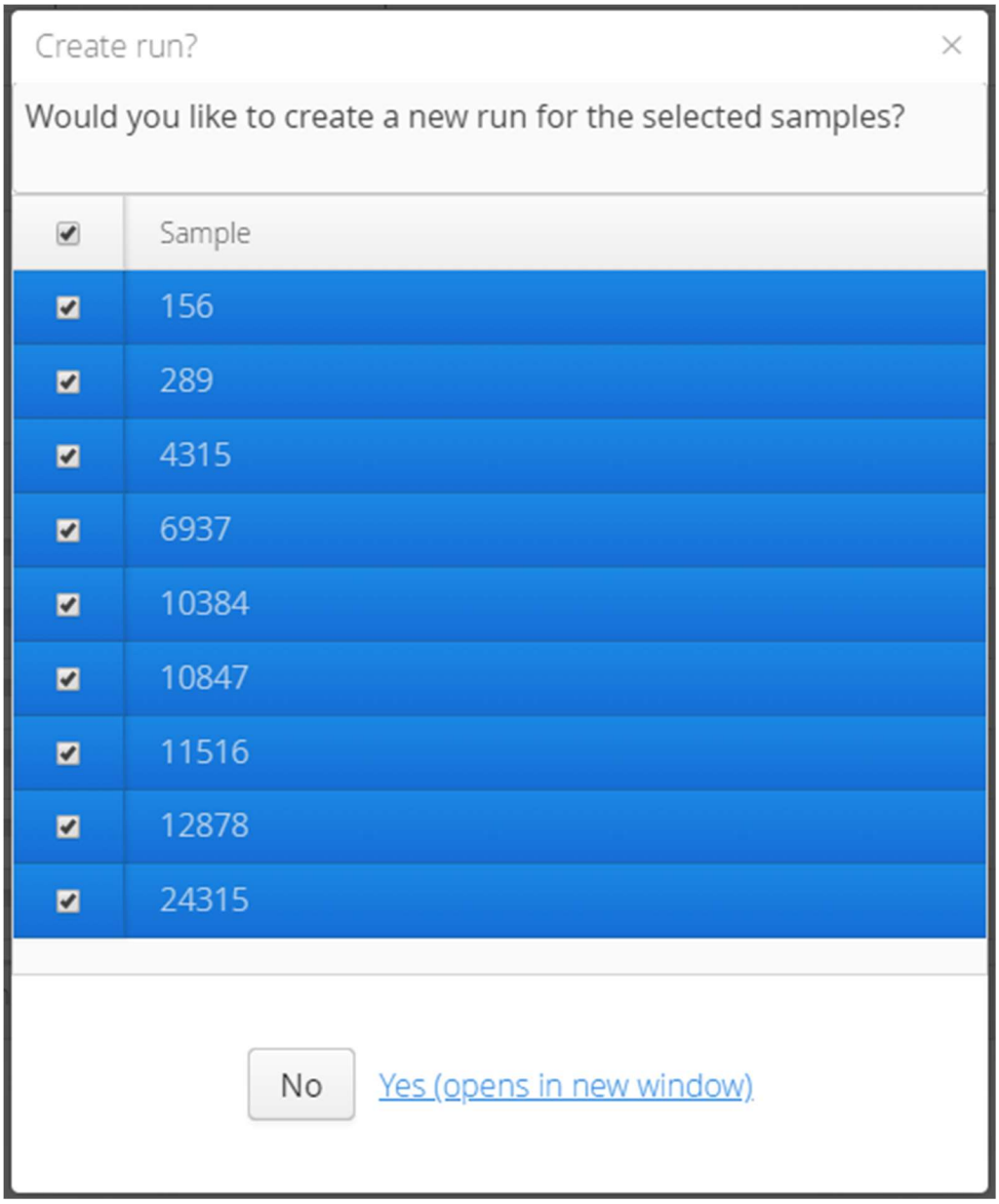
4. Click the **Select FASTQ Files** button, select all the FASTQ Files for any new reference or test samples, and click **Open**. The FASTQ files should be automatically paired and listed in the **Paired FASTQs** table.



5. Click **Upload Paired FASTQ Files**.



6. Click **Ok.**



7. Deselect any reference samples for which you do not want to generate CNV/LOH/SNV results, and click **Yes (opens in new window)**. **If using Chrome, separate the new tab into a different window and ensure that the Upload FASTQs tab is selected in the original window. This is essential to ensure that the upload continues in the background.**

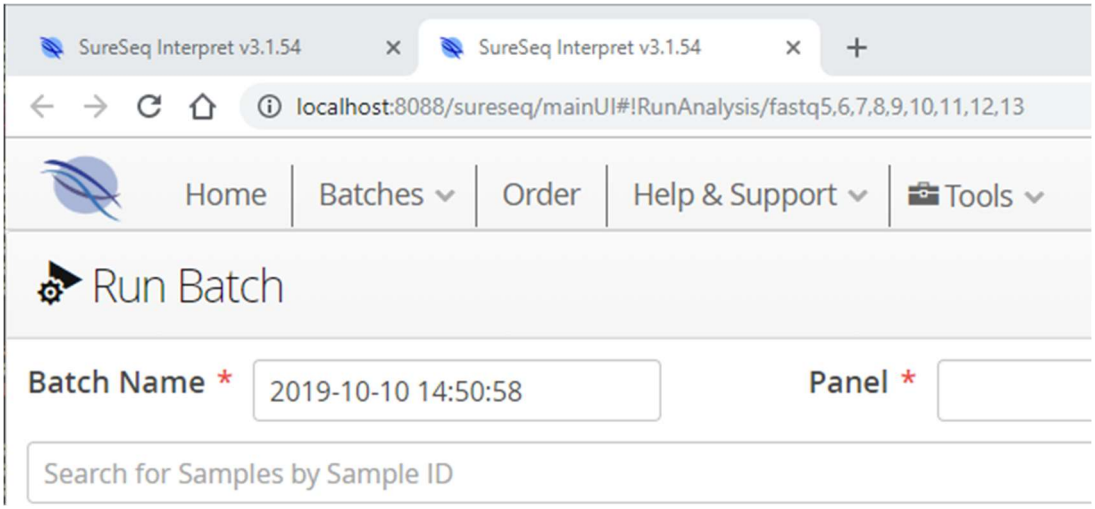


Figure 1: The **Upload FASTQs** tab is hidden behind the new tab in Chrome. Uploading is paused.

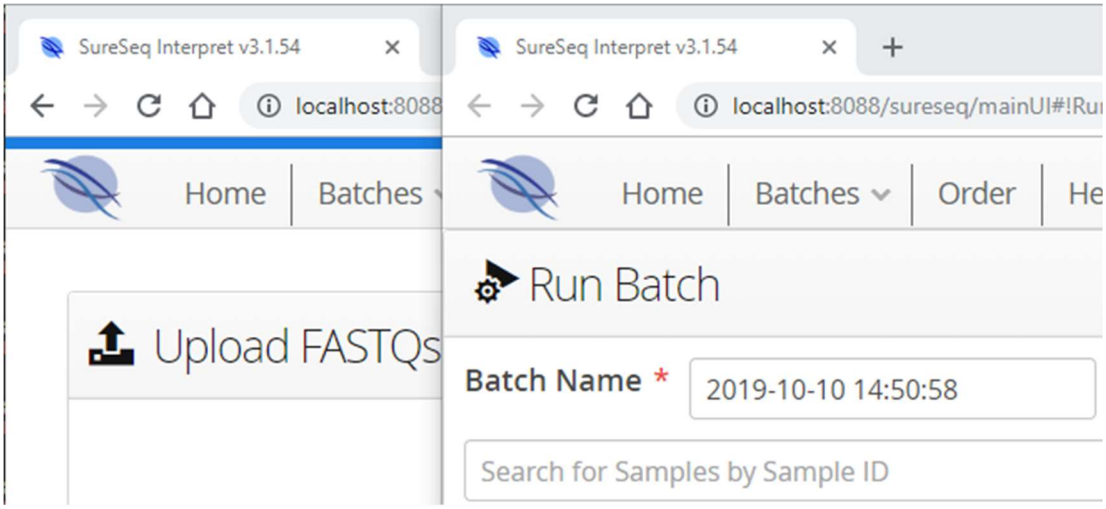
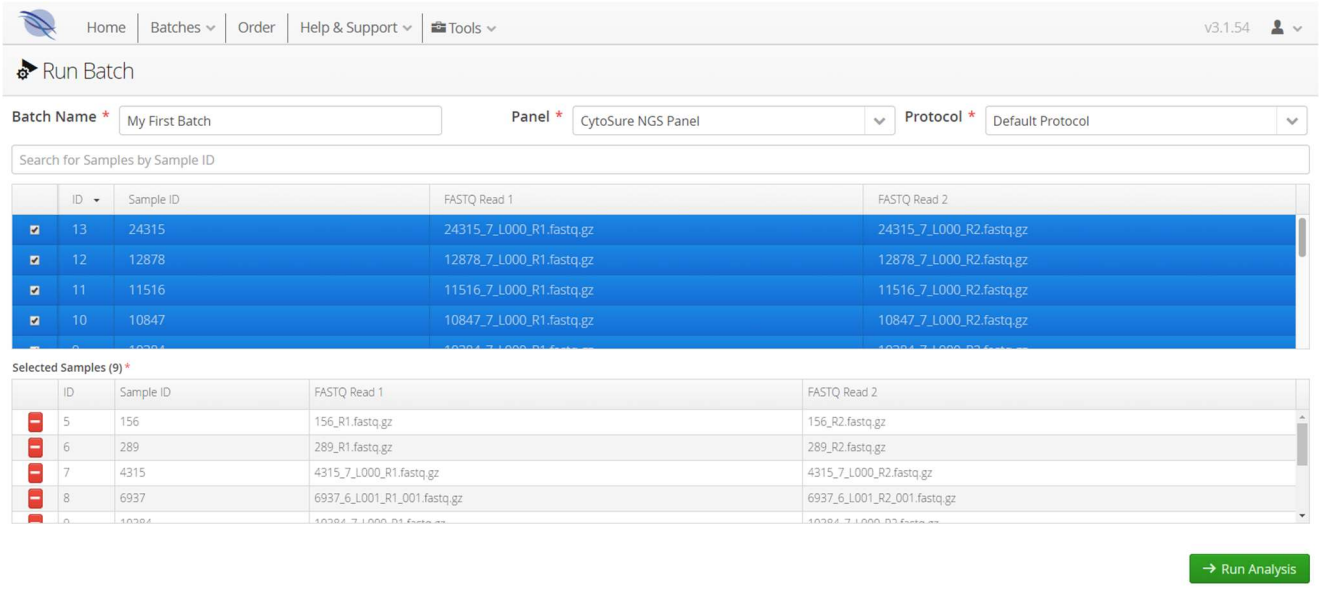


Figure 2: The new tab is separated from the **Upload FASTQs** tab. Uploading will continue in the background.

Start the Batch



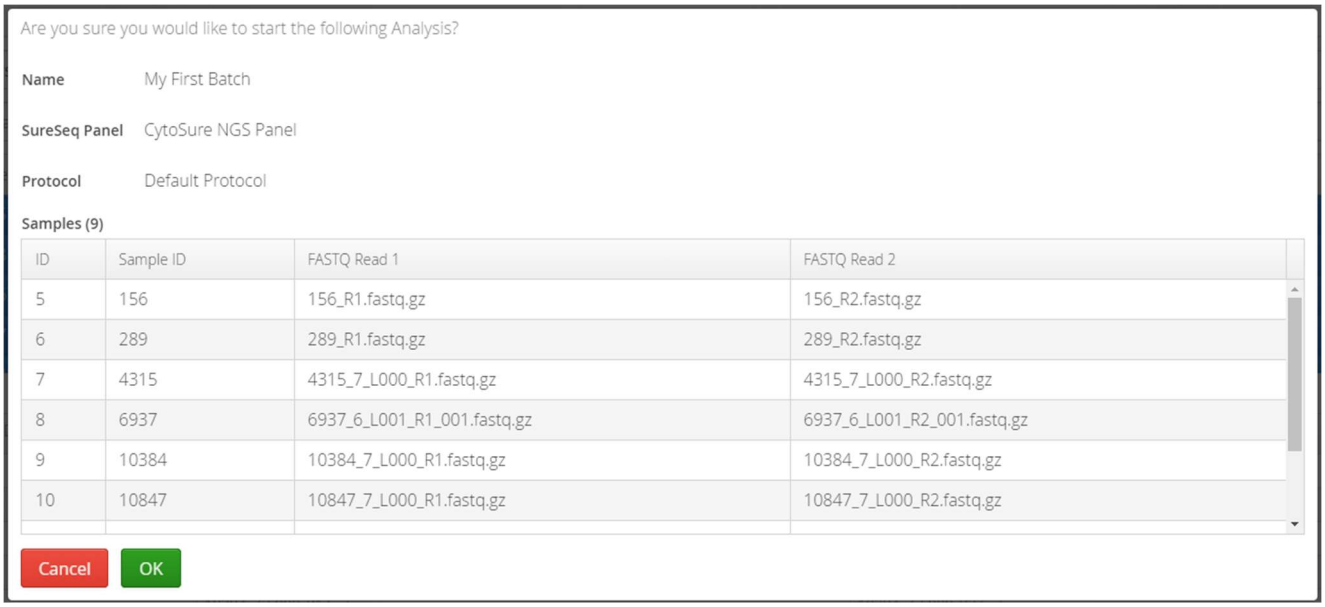
1. The samples previously selected for the batch should be listed in the **Selected Samples** table. If they are not, select the correct samples from the table above.

   1.1. Enter an appropriate name for the batch in the **Batch Name** field.

   1.2. Select the appropriate panel from the **Panel** drop-down box.

   1.3. Select **Default Protocol** from the **Protocol** drop-down box.

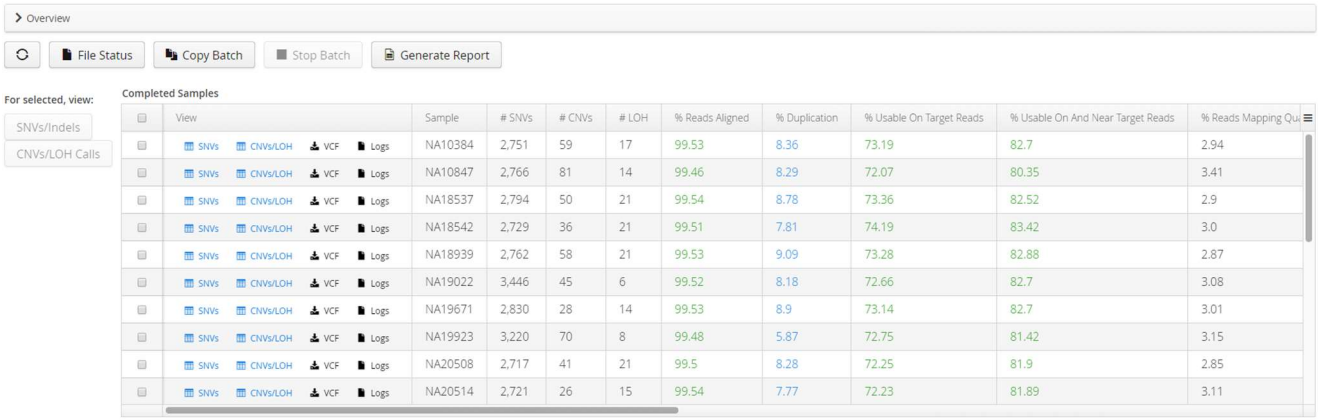
   1.4. Click **Run Analysis**.



2. Click **OK** to confirm that the details are correct and start the batch.

Monitor the Batch

The **Batch** view provides information on the progress of the analysis pipeline as it processes the samples in the batch. The status of both the batch as a whole and its individual samples is updated during processing so that progress can be monitored by the user.



The batch may be assigned the following statuses as it progresses, which are display in the **Status** field in the **Overview** section:

- **Waiting** - Waiting for the batch to be queued on the processing server, or for another batch to complete.
- **Transferring FASTQs** - Transferring the FASTQs of the test samples to the processing server.
- **Pre-processing Reference Samples** - Transferring and aligning the FASTQ files of the reference samples defined in the analysis protocol. The progress of each individual reference sample is also displayed.
- **In progress** - Running the pipeline on test samples.
- **Completed** - All samples have been processed successfully.
- **Failed** - The batch failed for at least one sample.
- **Killed** - The user forcibly stopped the batch.

If the batch doesn't appear to be progressing, or has failed, the **File Status** page provides the ability to download the logs for an individual sample which will help technical support with troubleshooting.

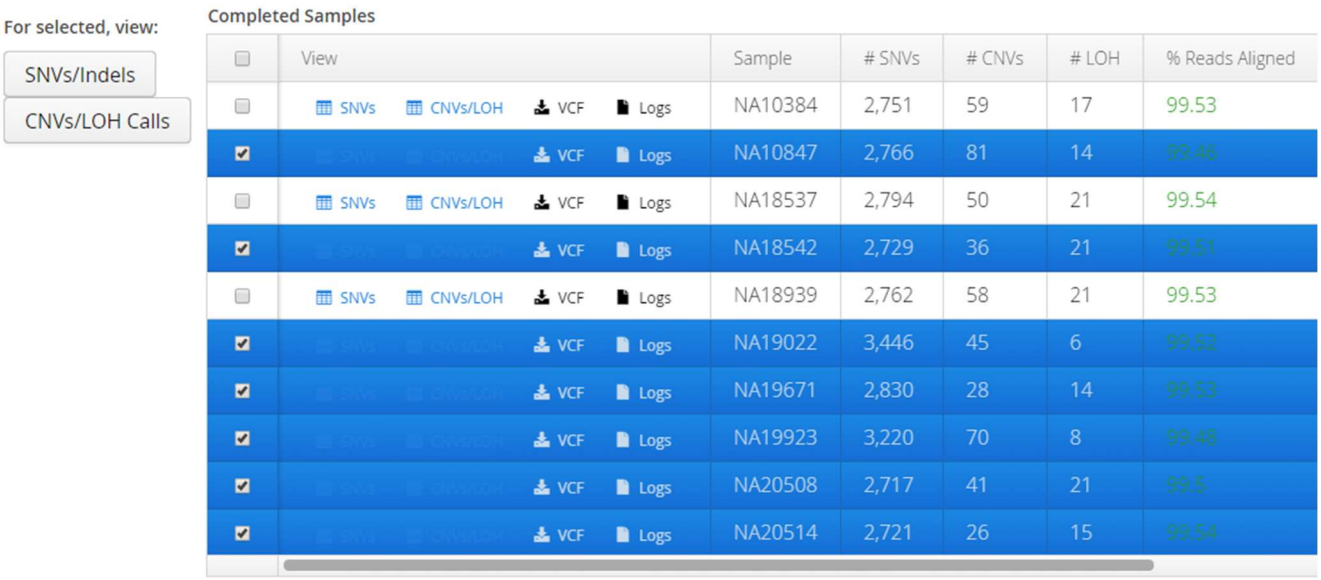
As each sample completes, an entry will appear in the **Completed Samples** table containing a summary of its QC metrics and variants detected.

View the QC Metrics

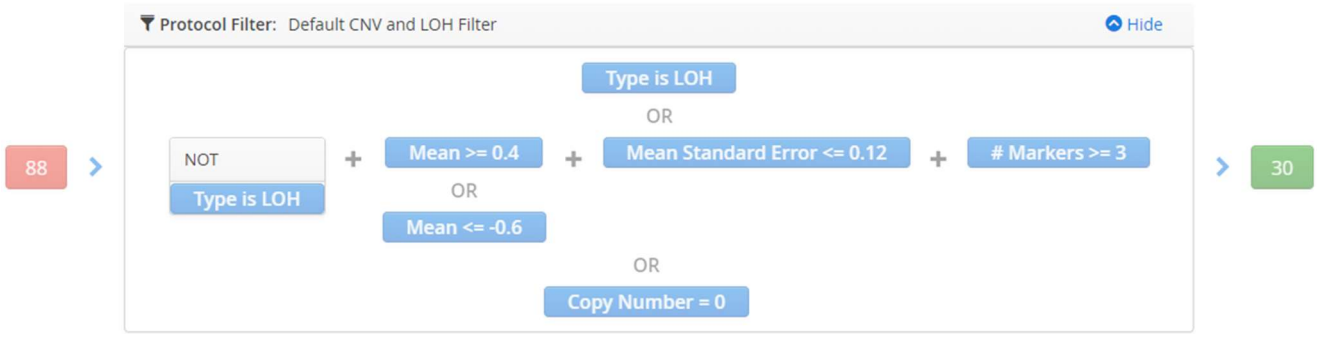
The **Batch** view provides access to QC metrics for the samples as follows:

1. In the **Completed Samples** table - The values of a number of QC metrics are presented to the user in the **Completed Samples** table. Some may be hidden from the table by default, but can be displayed by clicking on the  icon in the top-right corner of the table and selecting the missing columns.
2. In the "fastp" report - The **QC** column of the **Completed Samples** table provides access to the report generated by the "fastp" utility via the **View** button.
3. In the "MultiQC" report - The **Overview** section contains a link to the **MultiQC Report**, which includes various metrics and graphs of metrics for every sample in the batch in one HTML document.

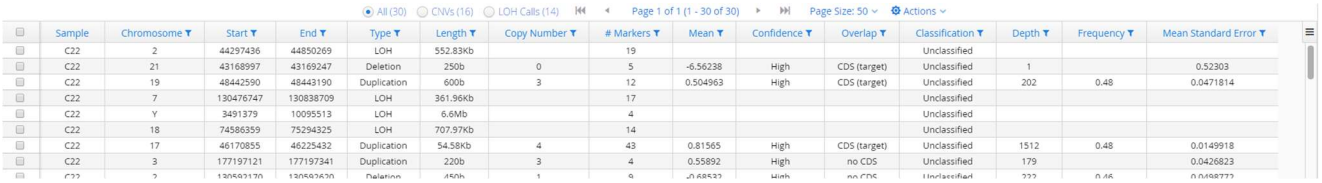
View the CNV/LOH Data



1. To view CNV/LOH data for a set of samples, select the corresponding checkbox for the sample in the **Completed Samples** table and click the **CNVs/LOH Calls** button. Alternatively, to view CNV/LOH data for an individual sample, click the **CNVs/LOH** in the **View** column for the sample.

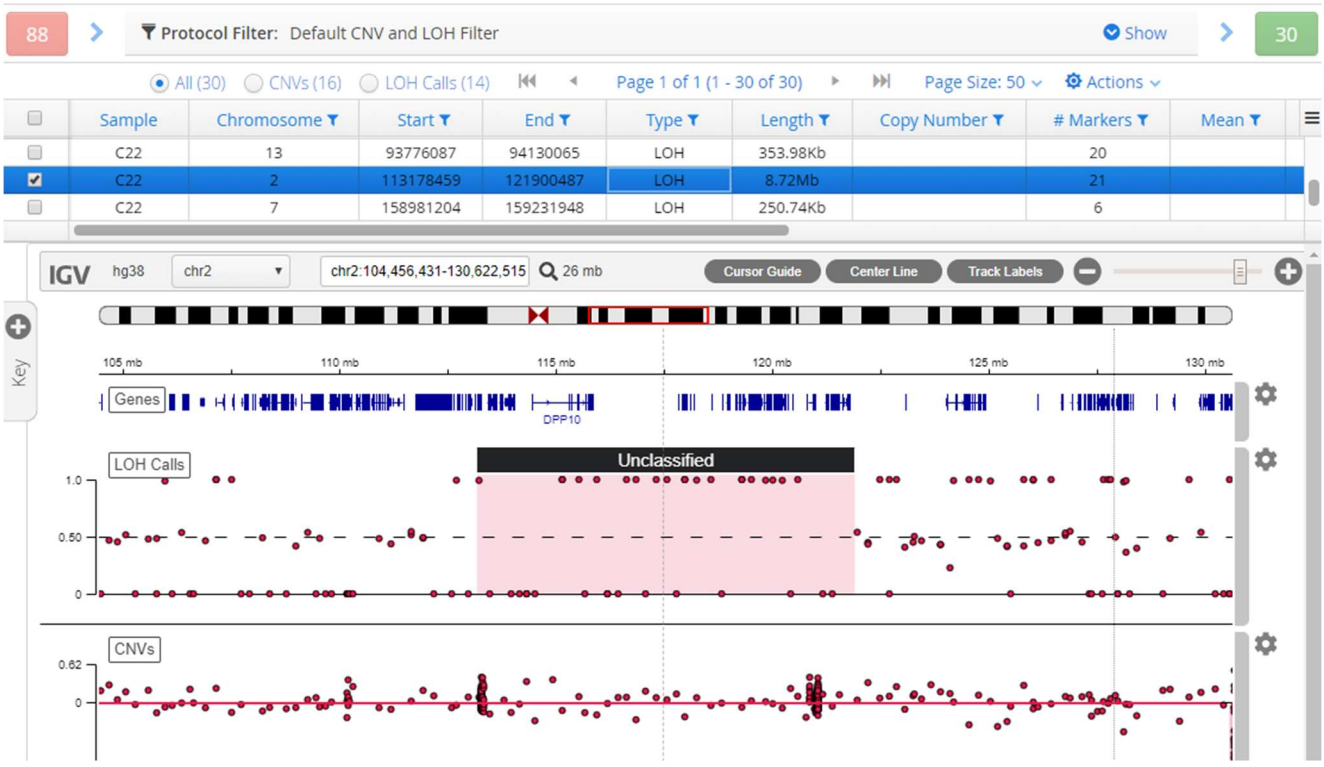


2. The **CNVs + LOH Calls** view should appear. The **Protocol Filter** section at the top displays the filters which have been applied to the detected CNVs and LOH Calls. This can be minimised by clicking the **Hide** button.



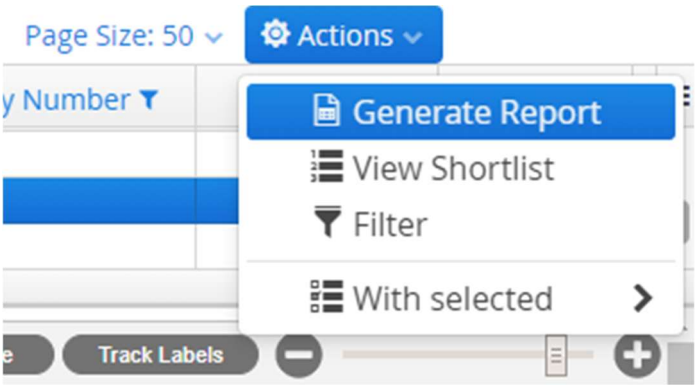
Sample	Chromosome	Start	End	Type	Length	Copy Number	# Markers	Mean	Confidence	Overlap	Classification	Depth	Frequency	Mean Standard Error
C22	2	44297436	44850269	LOH	552.83Kb		19				Unclassified			
C22	21	43168997	43169247	Deletion	250b	0	5	-6.56238	High	CDS (target)	Unclassified	1		0.52303
C22	19	48442590	48443190	Duplication	600b	3	12	0.504963	High	CDS (target)	Unclassified	202	0.48	0.0471814
C22	7	130476747	130838709	LOH	361.96Kb		17				Unclassified			
C22	Y	3491379	10095513	LOH	6.6Mb		4				Unclassified			
C22	18	74586359	75294325	LOH	707.97Kb		14				Unclassified			
C22	17	46170855	46225432	Duplication	54.58Kb	4	43	0.81565	High	CDS (target)	Unclassified	1512	0.48	0.0149918
C22	3	177197121	177197341	Duplication	220b	3	4	0.55892	High	no CDS	Unclassified	179		0.0426823

3. The CNVs and LOH Calls detected in the selected sample(s) are listed in the table in the middle of the page.

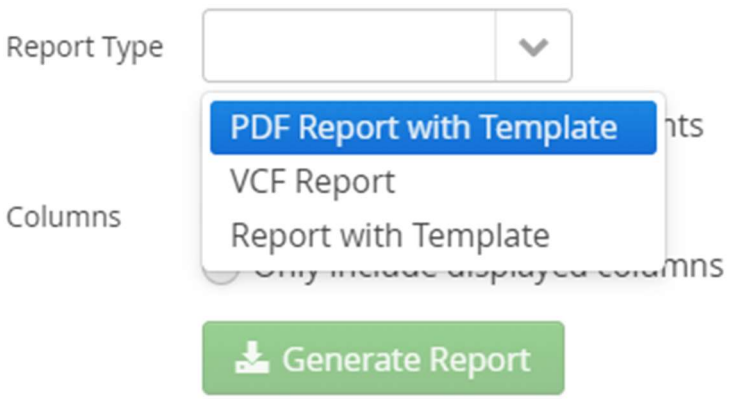


4. To view the genomic data contributing to a CNV or LOH call, click on the corresponding row of the table and the **IGV** section will be populated accordingly.

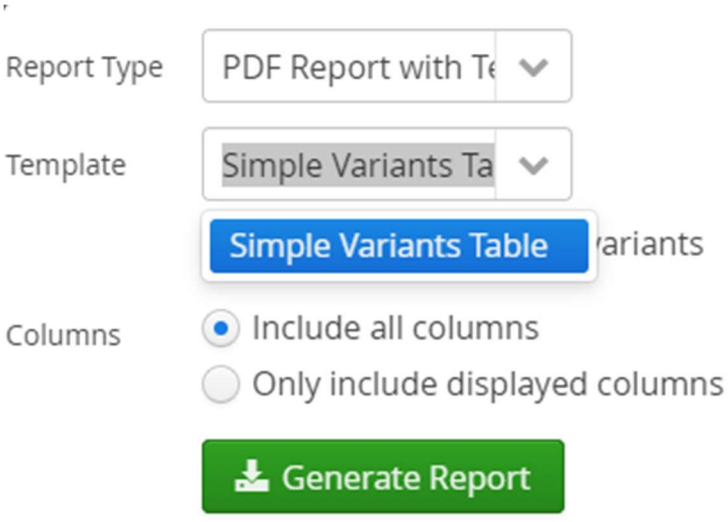
Generate a Report of the CNV/LOH Data



1. Select the **Actions** button above the CNV/LOH Calls table and click **Generate Report**.



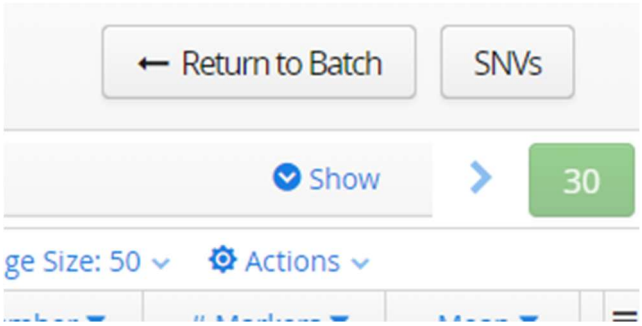
2. In the **Report Type** drop-down list, select the type of report you wish to generate.



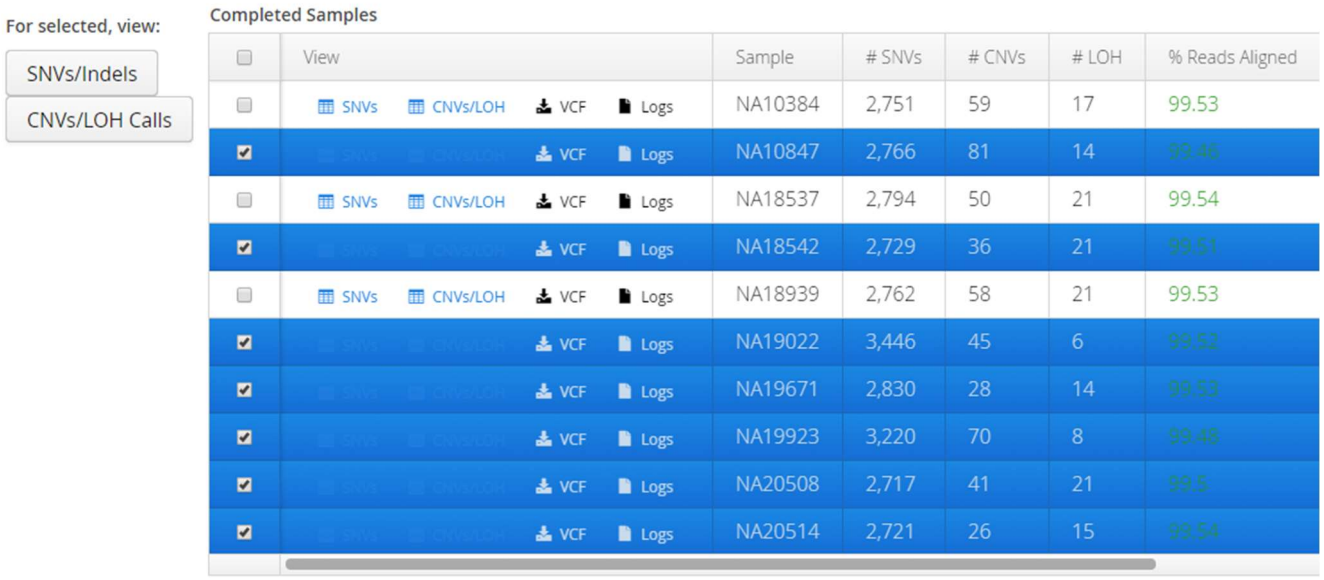
3. If necessary, in the **Template** drop-down list, select a report template.

4. Choose whether to **Include all columns** or **Only include displayed columns** and click **Generate Report**.

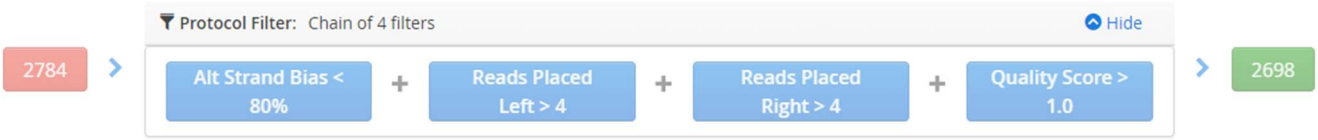
View the SNV Data



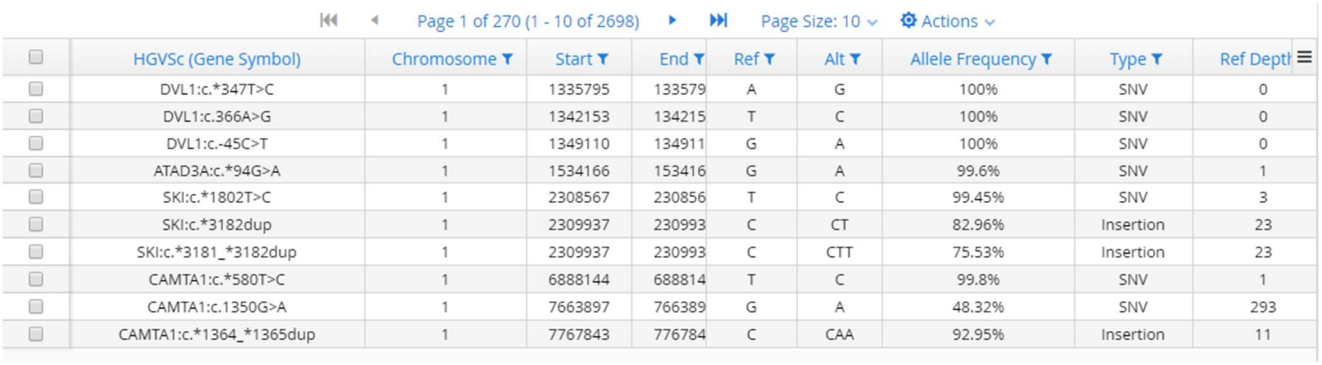
1. To view SNV data for the sample(s) currently displayed in the **CNVs + LOH Calls** view, click the **SNVs** button in the top-right corner.



Alternatively, click **Return to Batch**, select the sample(s) from the **Completed Samples** table and click the **SNVs/Indels** button.



2. The **Variants** view should appear. As with the **CNVs + LOH Calls** view, the **Protocol Filter** section at the top of the page displays the filters which have been applied to the detected SNVs/Indels, which can be hidden by clicking the **Hide** button.



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HGVSc (Gene Symbol)	Chromosome	Start	End	Ref	Alt	Allele Frequency	Type	Ref Depth
DVL1:c.*347T>C	1	1335795	133579	A	G	100%	SNV	0
DVL1:c.366A>G	1	1342153	134215	T	C	100%	SNV	0
DVL1:c.-45C>T	1	1349110	134911	G	A	100%	SNV	0
ATAD3A:c.*94G>A	1	1534166	153416	G	A	99.6%	SNV	1
SKI:c.*1802T>C	1	2308567	230856	T	C	99.45%	SNV	3
SKI:c.*3182dup	1	2309937	230993	C	CT	82.96%	Insertion	23
SKI:c.*3181_*3182dup	1	2309937	230993	C	CTT	75.53%	Insertion	23
CAMTA1:c.*580T>C	1	6888144	688814	T	C	99.8%	SNV	1
CAMTA1:c.1350G>A	1	7663897	766389	G	A	48.32%	SNV	293
CAMTA1:c.*1364_*1365dup	1	7767843	776784	C	CAA	92.95%	Insertion	11

3. The table in the middle of the page lists the SNVs/Indels detected in the selected samples(s). Additional information is available for display via the ≡ button.

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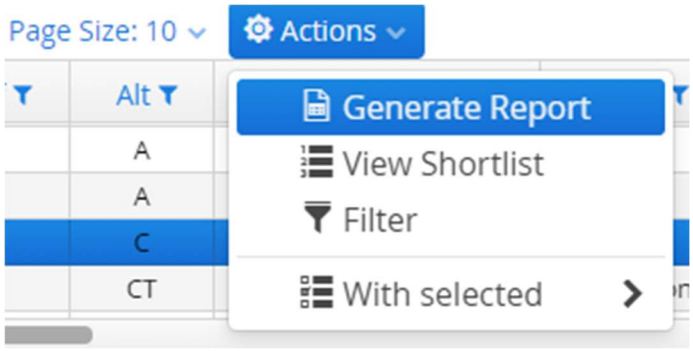
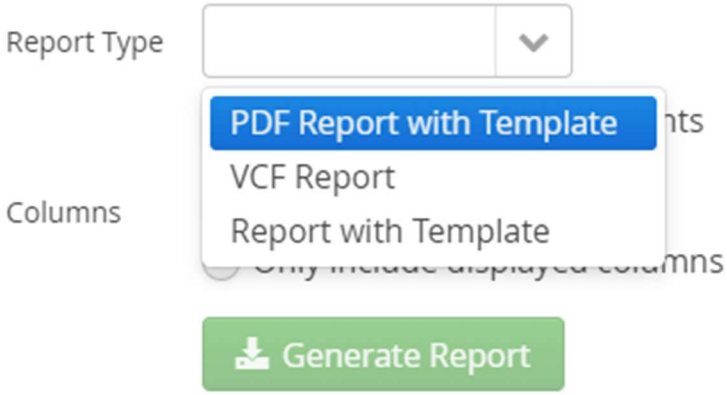
HGVSc (Gene Symbol)	Chromosome	Start	End	Ref	Alt	Allele Frequency	Type	Ref Depth
DVL1:c.-45C>T	1	1349110	134911	G	A	100%	SNV	0
ATAD3A:c.*94G>A	1	1534166	153416	G	A	99.6%	SNV	1
SKI:c.*1802T>C	1	2308567	230856	T	C	99.45%	SNV	3
SKI:c.*3182dup	1	2309937	230993	C	CT	82.96%	Insertion	23

4. To view the genomic data contributing to a SNV or Indel, click on the corresponding row of the table and the **IGV** section will be populated accordingly,

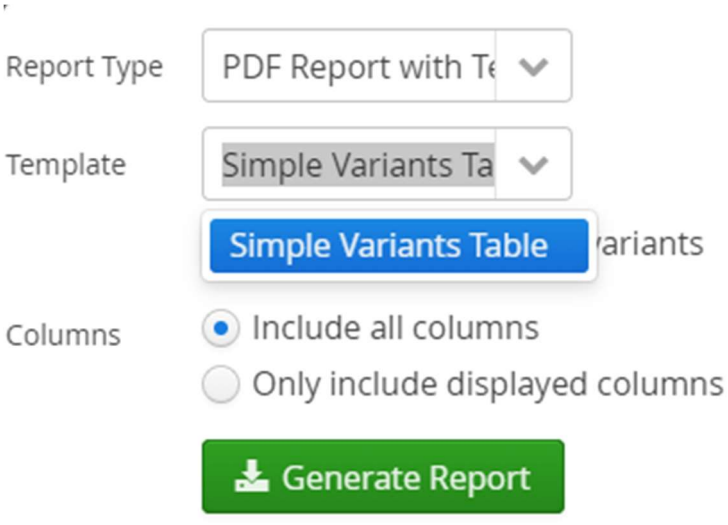
Generate a Report of the SNV Data



1. Select the **Actions** button above the SNVs/Indels table and click **Generate Report**.



2. In the **Report Type** drop-down list, select the type of report you wish to generate.



3. If necessary, In the **Template** drop-down list, select a report template.

4.Choose whether to **Include all columns** or **Only include displayed columns** and click **Generate Report**.

Contact us

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